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(54) Title: METHODS FOR SELECTIVELY REACTING LIGANDS IMMOBILIZED WITHIN A TEMPERATURE-SENSITIVE POLYMER GEL

(57) Abstract

Methods for delivering substances into, removing substances from, or reacting substances with a selected environment utilizing polymer gels or coatings characterized by a critical solution temperature (CST). The CST as well as the pore structure, pore size, pore distribution, and absorbing capacity of the gel may be selectively controlled. The substances may be physically or chemically immobilized within the polymer gels. In addition, a method for altering the surface wettability of CST polymers is also disclosed.

Description

"METHODS FOR SELECTIVELY REACTING LIGANDS IMMOBILIZED WITHIN A TEMPERATURE-SENSITIVE POLYMER GEL"

Cross-Reference to Related Application

This application is a continuation-in-part of U.S. application Serial No. 853,697, filed April 17, 1986 and assigned to Genetic Systems Corporation.

Technical Field

The present invention relates generally to methods for delivering substances into, removing substances from, or reacting substances with a selected environment in order to effect a desired purpose, utilizing polymer gels or coatings exhibiting either an upper or a lower critical solution temperature.

Background Art

A number of polymers undergo a phase separation in response to a change in environmental conditions, such as solution pH, ionic strength or temperature. For instance, some soluble polymers become insoluble when the solution temperature is changed only a few degrees. These polymers are said to possess a critical solution temperature (CST). A polymer possessing a lower critical solution temperature (LCST) becomes insoluble when the temperature of the solution is increased through a particular narrow temperature range. Conversely, a polymer possessing an upper critical solution temperature (UCST) becomes insoluble when the temperature of the solution is decreased through a particular narrow temperature range.

Polymers capable of phase changes in response to temperature changes have been described by Taylor in U.S. 3,427,892 for use in controlling a process relating to film developing. In Taylor, a layer of polymer changes permeability as a function of temperature. The polymers are

mammalian body, for example. The gel is said to release insulin into the body over time as it diffuses through the gel pores.

Cussler, in U. S. Patent No. 4,555,344, describes using a cross-linked ionic polymer gel, such as partially hydrolyzed polyacrylamide or dextran, to selectively absorb a low molecular weight solvent and solute from a solution that includes higher molecular weight components in the solution. The gel is introduced into the solution in a shrunken state. A pH change or a change in composition of the solution is required to cause the gel to rapidly swell in volume, absorbing low-molecular weight solvent and solute.

Graham, in U. S. Patent No. 4,584,188, describes gels comprising a polymerizable cyclic (thio) ether and a hydrophilic homo- or copolymer. A temperature change is required for expelling or releasing from the gel an active substance previously absorbed from a solution.

A limitation to date regarding the use of polymer gels that change phases in response to a change in environmental conditions has been that separations from or deliveries to solutions have been substantially nonspecific. Both the Cussler and the Graham inventions rely upon a temporary physical entrapment of the solution or solvent within the gel. That is, a CST gel, for example, in response to a change in temperature through the CST absorbs a liquid to which it has been exposed. The solution is absorbed by the gel nonspecifically, only excluding molecules too large for its pore structure.

Consequently, there exists a need in the art for an improved system for controlling biological or chemical reactions in selected environments by providing methods of separating certain desired substances from a solution, delivering certain selected substances to, or exposing certain selected substances to a desired environment, which methods are readily and efficiently controllable. The

with the substance desired to be delivered to the selected environment. The temperature of the polymer gel is adjusted, whereby the gel absorbs the substance. The gel is placed into contact with the environment of interest and the temperature adjusted, whereby the gel releases the substance into the environment. As noted above, the initial temperature of the polymer gel may be such that it is in a partially or totally desolvated state when contacted with the substance to be absorbed and subsequently delivered. Alternatively, the polymer may be in a solvated state, contacted with the substance, and the temperature cycled, causing the polymer to desolvate and, upon reversing the temperature, to solvate, absorbing the desired substance.

The substance may be incorporated in the polymer by physical absorption or entrapment within the pores of the gel. The substance may also be incorporated or entrapped via molecular entanglements and interactions within denser regions of the polymer gel, typically by means of secondary (ionic, polar, hydrophobic, etc.) forces binding the substance to the polymer. In addition, the substance may also be bound to the gel matrix by means of labile, primary covalent bonds. A labile bond arrangement is useful in drug delivery, wherein, for example, an LCST gel polymer at 37°C is desolvated and includes the drug bound to the polymer by means of the labile bond. The gel is injected into the body in the form of gel particles or microspheres including the drug. The desired delivery area is then cooled to 35°C, which causes the gel to swell and absorb surrounding aqueous solution, whereupon the drug polymer bond is broken and the drug released.

The pore structure of the CST polymer may be adjusted by a number of means to selectively retain a desired substance within the gel. Pore structure, and hence, the amount of substance released and release rate are selectively controlled by adjusting the composition and/or concentration of monomers in the synthesis mixture employed to form the gel. Also, adjusting the amount and

tially hydrophobic N-substituted acrylamides or methacrylamides), hydroxy alkyl celluloses, polyoxazolidones, polyvinylmethylether, polyethylene oxide, polymethacrylic acid, or copolymers thereof, including variations in the polar or hydrophobic components of all of these polymers.

The methods described herein may also include a polymer gel that is characterized by an upper critical solution temperature (UCST). Such a polymer gel may include a polymer such as polyacrylic acid, polymethacrylamide, or polyvinyl alcohol, and copolymers thereof.

The methods described herein, in addition to delivery, allow the separation of a desired substance from a solution. Typically, the procedure is initiated by introducing a dry or shrunken polymer gel characterized by a critical solution temperature (as described above) into the solution containing the desired substance. Utilizing the CST character of the polymer, one can adjust the temperature of the gel/solution sufficiently to cause the gel to swell and absorb the desired substance. As with the delivery method, the process may utilize either a lower or upper critical solution temperature polymer of the type described above.

This separation method is particularly useful in bioseparations, wherein the polymer gel includes a first component of a binding pair bound physically or chemically to the polymer. The polymer gel/binding component is contacted with a solution that contains the second component of the binding pair. This mixture is then incubated at a temperature sufficient to cause the polymer gel/first binding component to absorb liquid from the solution containing the second binding component, thereby allowing the second binding component to specifically bind to the first binding component. The solution is then heated or cooled to cause the gel to shrink and desolvate whereby the gel releases the remaining solution and retains the bound binding pairs.

ciently to allow solution containing the enzyme substrate from the environment of interest to contact the enzyme, thereby initiating and catalyzing the reaction of interest. Further adjustment of the temperature allows one to control the rate of reaction. Reversing the temperature beyond a CST will cause the polymer gel to shrink, closing off contact of the reactant with the solution, thereby substantially slowing down or terminating the reaction. Alternatively, the reaction may be selectively controlled by gradually causing the polymer gel to shrink. In addition, through use of a combination of temperature and controlled pore size, one can selectively eliminate access of different sized molecules as the temperature is raised or lowered.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings.

Brief Description of the Drawings

Figure 1 presents vitamin B_{12} delivery as a function of cross-linker/monomer ratio, for indicated solution, monomer and solvent composition used to synthesize poly-NIPAAm gels.

Figure 2 depicts myoglobin delivery as a function of cross-linker/monomer ratio for various NIPAAm polymer gels synthesized by the indicated solution, monomer and solvent compositions.

Figure 3 illustrates the delivery of vitamin B_{12} as a function of time for three different polyNIPAAm gels.

Figure 4 demonstrates the impact on water contents vs. temperature of copolymerizing methacrylic acid with NiPAAM to form LCST polymer gels.

Figures 5 (a-c) depict the release of methylene blue at 20°C and 50°C as a function of time for NIPAAm polymer gels copolymerized with up to 5% methacrylic acid.

Figure 6 depicts the release rate, as a function of temperature, of methylene blue from a cross-linked

Best Mode for Carrying Out the Invention

The methods of the present invention utilize, as a principal component, a polymer that is characterized by a capability of precipitating from an aqueous solution within a narrow temperature range. The temperature at which polymer precipitation is initiated is often termed the "critical solution temperature" (CST) for the polymer.

The CST polymer of interest may be copolymerized and/or cross-linked to form a gel which absorbs or releases a liquid or vapor in response to a temperature change at the CST.

Polymers having a CST are well known in the literature. See, for example, Molyneux, Water-Soluble Synthetic Polymers; Properties and Behavior, CRC Press, Boca Raton, Florida (1983); Finch, C. A., ed., Chemistry and Technology of Water-Soluble Polymers, Plenum Press, New York (1983) at 163. A combination of hydrophobic and hydrophilic components may be selected to form a desired CST characteristic.

The majority of CST polymers exhibit phase separation or precipitation upon cooling and are referred to as including an upper critical solution temperature (UCST) characteristic. A number of polymers of interest, however, exhibit precipitation upon heating. The temperature at which precipitation occurs is referred to as the lower critical solution temperature (LCST) of the polymer.

Polymers having upper critical solution temperatures (UCST) include many diverse polymers. A few aqueous systems include polyacrylic acid, polymethacrylamide and polyvinyl alcohol. Polymers that exhibit a lower critical solution temperature (LCST) include polyethylene oxide, polyvinylalkylethers, polyvinylmethyl oxazolidone, polymethacrylic acid, substantially hydrophobic N-substituted acrylamide polymers, hydroxy alkyl celluloses as well as alkylcelluloses. See Finch, C. A., ed., Chemistry and Technology of Water-Soluble Polymers, Plenum Press, New York (1983) at 157.

character, likewise may have a major impact upon gel pore structure as well as LCST or UCST.

Careful choice of ingredients and the synthesis parameters noted above allow production of a gel that is capable of selectively retaining or excluding molecules on the basis of size. Relatively lower levels of cross-linking agent for a given monomer will result in larger pore sizes. Where self-supporting gels are desired, however, pore size increases effectuated by means of reducing cross-linker are limited by the mechanical strength the gel must retain in order to hold together. For larger pore sizes with the same monomers, the gel may be incorporated within (another polymer or), attached to or applied as a coating on a supporting surface or base. The polymer gel coating could be attached chemically or by means of plasma discharge, ionizing radiation, UV process, ozone process or the like. The gel may be cross-linked during or after processing using a variety of means to effect the cross-linking, including incorporation of cross-linkers during the coating or incorporating process as well as the use of cross-linking compounds, ionizing radiation or UV after the system is polymerized.

The selection of cross-linking agents is one of the determining factors in achieving a desired gel pore size, which will be important for larger substrates or other reactants. The cross-linker may be hydrophobic or hydrophilic. The hydrophobic cross-linker may be any short di- or trivinyl or di- or triallyl monomer, such as methylene bis-acrylamide. A hydrophilic cross-linking agent may be, for example, a long-chain polyethylene glycol, both ends of which include double bonds for binding to the polymer backbone. Thus, the choice of cross-linker permits covalently bonding into the polymer gel hydrophobic or hydrophilic groups, which will effect the pore size and its distribution.

The concentration and composition of the initiators used in a free-radical polymerization can also

over which the separation (absorption) or delivery (desorption) occurs. Such an extended response may be desired, depending upon the particular environment of interest.

Including a second CST polymer in the gel polymerization mixture produces a gel which has two CSTs, each reflecting the character of its backbone polymer composition. An interpenetrating network (IPN) of the two polymers is formed. Thus, water or solution evolution from a gel in a delivery process for such an IPN gel will show two distinct drops in swelling solution content as temperature is increased through the first and then the second LCST.

As noted above, the present invention allows the immobilization of a ligand within the gel in a manner such that the ligand may be exposed to and/or isolated from an environment through the gel's CST. The term "cycling", as used herein, means adjusting the temperature of the CST polymer gel through the CST range, whereby the gel becomes solvated, followed by desolvating, or vice versa, as the CST is passed.

A ligand (binding pair) immobilized within the gel may be cycled through the gel CST to imbibe solution from an environment and bind a substance of interest. Further temperature cycling will expel unbound substance/solution. The ligand bound substance may then be released from the immobilizing binding pair component by further cycling the gel in an eluting solution that breaks either the bond to the polymer gel or the binding pair bond. The substance of interest may then be expelled from the gel. The eluting solution may effect the breaking of the bonds or linkage by a change in pH, the presence of certain ions and ionic concentrations, the presence of hydrogen bonders, certain solvents, water structurers, destructurers, oxidants or reductants, reactants, affinity ligands and the like.

As noted above, a substance may be delivered to or removed from an environment by employing a ligand that

one may immobilize a drug, an antibody, or other biomolecule to the backbone of this gel via such linkages, and keep the gel in a dry or shrunken state. When such a gel is immersed in a solution below its LCST, it will swell and imbibe solvent, and if the solvent has the capability of degrading the linkage, then the drug (or antibody/antigen complex) or other biomolecule will be released from the backbone of the gel, and upon subsequent warming may be released from the gel into the surrounding environment as the gel collapses above its LCST.

The ligand immobilized (physically and/or chemically) within the polymer gel may be a binding component of an affinity binding pair. Suitable affinity binding pairs include an antibody which binds with an antigen or hapten of interest. A receptor may be bound to the polymer gel that is designed to bind with a hormone, vitamin, lectin, drug, dye or lipid binding partner in solution. Other ligand binding pair components include lectin and polysaccharide or glycoprotein; DNA, RNA (single- or double- stranded) with complementary DNA, RNA or oligonucleotides or proteins or steroids; ion with chelator, ionophore, complexer; and stable-free radical with free radicals.

Suitable ligands for immobilization within the gel may also include a nonspecific binding component that is suitable for reacting with a binding partner of interest in an environment. For example, an anion or polyanion could be incorporated or immobilized within the polymer gel and bind, via ionic bonding, with a cation or polycation in the environmental solution. An anion and cation pair in the environmental solution could bind with a polyanion/polycation complex within the gel. One may immobilize a lipid or hydrophobic ligand within the polymer gel which would bind via hydrophobic bonding with a lipophile in solution. Acid/base-type interactions could be used to affect binding by immobilizing an electron donor which

swollen gel to shrink noticeably and become somewhat hard or brittle. These mechanical and/or dimensional polymer gel changes can be used to affect mechanical movements that serve to signal a temperature change. Conversely, a temperature change can be used to affect mechanical or dimensional changes.

Wettability of the surface of the CST polymer gel changes as its temperature changes through the gel CST. An LCST polymer gel loses wettability as the temperature is raised through the LCST. These changes are observable by means of conventional contact angle measurements. In addition, the wettability phenomenon is important for surfaces which are in contact with reactant solutions. Surfaces may be wetted or dewetted as the temperature is varied above the CST for a desired effect. For instance, the wetting or dewetting could be used as a signal to transfer heat into or out of the system, or could be used to eliminate fouling components from a surface.

The gels of the present invention also change optical characteristics at the CST. For example, increasing a gel temperature above its LCST causes the gel to become opaque. If an enzyme is immobilized within a gel and is included in a membrane system that is viewed optically, a temperature increase to an undesirable level could be detected by a change in the optical transmission of the gel membrane. This change can be used to generate a signal providing feedback to a coolant system that turns on and off in response thereto.

The CST polymer gel materials may also be employed in a variety of forms. For example, the materials may be utilized as films or membranes, tubes, hollow fibers, solid fibers, fabrics (woven, knit or non-woven), molded objects, solid particles, capsules, polymeric micelles or liposome-like structures. Likewise, they may be applied as coatings on solid surfaces or in the pores of porous solids, as solutions, particulate suspensions, etc. Coatings may be

biologic aggregates such as organelles and whole cells themselves. Living cells contain many enzymes and can be immobilized within LCST polymer gels such that when the temperature is warmed above the LCST, the polymer gel will shrink and squeeze out the fluid within the pores. This aqueous solution could contain the product secreted from the cells, and then would potentially enhance the recovery of that product by this rapid delivery process. Additionally, when the gel is reswollen below the LCST, this would enhance the rate of mass transfer of nutrients, enzyme substrates and other reactants, and oxygen to the cell, which it requires in order to synthesize and secrete the product of interest. Thus, a temperature cycling in such an immobilized cell system could provide both enhanced yields and enhanced rates of production of specific biological products of enzymatic processes within living cells.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE I

A METHOD OF MAKING A THERMALLY REVERSIBLE GEL OF POLY-N-ISOPROPYL ACRYLAMIDE (NIPAAm)

A solution of N-isopropyl acrylamide monomer in dimethyl sulfoxide (DMSO) was prepared at various monomer concentrations. Methylene bis-acrylamide (MBAAm) was added as a cross-linker to the solution at various cross-linker ratios of MBAAm/NIPAAm. Benzoyl peroxide (BP) was added at a constant ratio of 0.001 mole BP/mole NIPAAm. The solutions were sparged with N_2 and kept over N_2 at room temperature. Then N,N dimethyl toluidene (NNDMT), a co-catalyst with BP, was added dropwise with stirring until the ratio of NNDMT/BP = 1 was reached. The solutions were each then poured quickly between glass plates spaced 0.75 mm apart and sealed at the edges. The plates were immersed in cool water and polymerization allowed to proceed 30-60 minutes. The plates were separated, the gels removed and

having a molecular weight of 17,800, and a like amount of vitamin B_{12}, having a molecular weight of 1,350. The samples were incubated at 4°C overnight. The films were then removed from the original solution, quickly rinsed in room temperature buffer and then deswelled in 10 ml of warm buffer at 50°C for 4 minutes.

Concentrations of the myoglobin and vitamin B_{12} released were determined by absorption at 280 nm and 360 nm, respectively. Figures 1 and 2 report the weight ratio of myoglobin or vitamin B_{12} to buffer released, reported as a function of cross-linking agent/monomer ratio densities. Comparing polymer gels made with 20% NIPAAm monomer in water with the 20% NIPAAm polymerized in DMSO shows that the water-synthesized 20% NIPAAm polymer absorbed and delivered myoglobin while the DMSO-synthesized 20% NIPAAm gel did not. Referring to Figure 2, vitamin B_{12}/buffer release as a function of cross-linker to monomer ratio is reported. Figure 2 reports that both the 20% NIPAAm gels, whether polymerized in DSMO or in water, absorbed and delivered vitamin B_{12}.

The comparison of the Figure 1 and 2 curves for 20% NIPAAm demonstrates that LCST hydrogels are capable of distinguishing, with respect to absorption and delivery, on the basis of molecular size. The example demonstrates that selection of important synthesis factors, such as cross-linker to monomer ratio and synthesis solution composition, may be used to affect the removal or delivery of molecules or substances from or to the environment.

Referring to Figure 3, the ratio of vitamin B_{12} to buffer released on heating polyNIPAAm gels from 4°C to 50°C as a function of time is reported. Release kinetics of vitamin B_{12} from various gels show two regions over time. The first region, occurring within the first five minutes of the temperature change, is a relatively sudden release of the solution nearest the surface of the gel and retained within its pores. The region thereafter shows a much slower diffusion rate out of the gel after the initial

content also has a significant influence on water content at higher temperatures. As shown in Figure 4, some gels have passed through their LCST, while others have not reached their LCST. It is possible that at high enough comonomer contents the gel will not exhibit an LCST up to 100°C in water.

EXAMPLE IV

METHYLENE BLUE ABSORPTION AND DELIVERY AS A FUNCTION OF METHACRYLICACID CONTENT OF NIPAAm POLYMER GELS

Gel polymer samples were made in accordance with Example III above, in which 0-5% methacrylic acid, in separate samples at 1% increments, was copolymerized with the poly-NIPAAm polymer. Methylene blue was absorbed into those samples in order to determine delivery capabilities as a function of amount of methacrylic acid incorporated within the gel.

The dried gels were equilibrated in a dye solution of 1% methylene blue dissolved in a 50/50 methyl alcohol and a 0.1 M Tris buffer (pH 8.61) solvent for 24 hours at room temperature. The polymer gel is dipped in a buffer at pH 7.4 at 20°C for 1-2 seconds to wash off excess solution on the surface. The gel is then placed in a buffer to release the methylene blue at temperatures of 20, 30, 35, 40 and 50°C. The amount of methylene blue released into the solution was determined using a UV-visible spectrophotometer. The following table reports the methylene blue initially absorbed into the gels.

A comparison of the release rates of methylene blue into aqueous buffer or distilled water from NIPAAm polymer gels including 1% methacrylic acid was conducted. The results, as depicted in Figure 6, show that including buffer salts in the surrounding solution made it easier to deliver methylene blue on heating above the LCST. This occurs because the salts break the negative/positive ionic bond between the negatively charged methacrylic acid component of the gel and the positively charged methylene blue. Thus, the buffer is acting as an eluting solvent. This action is important in both release and recovery of many binding partner solution components. Other systems of interest include, for example, assaying antigen in an immunoassay or recovering a product such as a peptide vaccine (which acts as an antigen to an immobilized antibody), when using these gels for bioseparations. The first binding partner, e.g., antibody, may be immobilized within the gel by including it in the polymerization solution as the free binding partner, or as the monomer conjugated binding partner. The immobilized binding partner may be any of the specific or nonspecific binding partners, as listed above.

EXAMPLE V

ASPARAGINASE ENZYME ACTIVITY AFTER IMMOBILIZATION WITHIN NIPAAm AND NIPAAm-METHACRYLIC ACID POLYMER GELS

In this procedure, 5 ml of 20% NIPAAm solution in 0.1 M Tris buffer (pH 8.6) were mixed with various percentages of methacrylic acid, as noted above. The solution was combined with a MBAAm cross-linker in the ratio of one mole of cross-linker per 750 moles NIPAAm. To this solution, an oxidizing redox catalyst component, comprising 10 microliters of 10% ammonium persulfate solution in distilled deionized water, was added. The mixture was degassed under vacuum and blanketed with nitrogen. 0.5 ml of asparaginase enzyme solution in 0.1 M Tris buffer was added to the

The data presented in Figures 7-10 show that the immobilized enzyme activity may be "turned off" by heating the polymer gel above its LCST, whether the enzyme is incorporated as free enzyme in the gel or as a monomer-conjugated enzyme bound to the polymer gel during preparation of the gel. The curves show that the presence of 1% methacrylic acid shifts the LCST and thus the "turn off" temperature. Further, the data demonstrate that the "turn off" mechanism is reversible, i.e., that the enzyme retains activity during a number of temperature cycles. The gels demonstrate that such reversibility is possible for some gels, such as NIPAAm containing 1% methacrylic acid, while not in others, such as NIPAAm containing no methacrylic acid. It appears that the enzyme may be denatured when the NIPAAm gel is cycled to 50°C, but remains active when the gel includes a methacrylic acid component, possibly due to the higher water content of the methacrylic acid-containing gel and/or to the local pH within the gel. The temperature combined with the composition of the gel may act together to denature the enzyme.

Figure 11 shows the water content of polymer gels that include asparaginase, immobilized therein. These data show that the presence of enzyme in gels does not effect the water content versus temperature curves in a buffer. The enzyme may be incorporated as free enzyme or be bound onto the backbone by its monomer conjugation during gel formation. A major and controlling factor demonstrated by these data is the impact of the monomer, in this example, methacrylic acid, and its concentration.

EXAMPLE VI

ASPARAGINASE ENZYME ACTIVITY AFTER IMMOBILIZATION WITHIN NIPAAm AND AAm POLYMER GELS

N-succinimidyl methacrylate (NSMA) dissolved in dimethylformamide was combined with a solution of asparaginase in 0.1 M Tris buffer, pH 8.6, for a total monomer:enzyme ratio of 91.7:1. This mixture was allowed

Table: EXAMPLE VI

Sample Code	Relative Amount(%) NIPAAm	AAm
NA-100	100	0
NA-95	95	5
NA-90	90	10
NA-85	85	15

Enzyme activity was measured at varied temperatures between 20° and 60°. For these measurements, the gels were first removed from storage at 4°C and equilibrated at room temperature for 1 hour. The gels were reacted for 10 minutes at room temperature, equilibrated at 30°C for 15 minutes, then reacted for 10 minutes in asparagine solution at 30°C. This pattern of equilibration for 15 minutes, then reaction for 10 minutes was repeated for each temperature studied. The specific enzyme activities of the gels are shown as a function of increasing temperature in Figure 12. It can be seen that the enzyme activities parallel the water contents of the gels. Thus, the activity of the immobilized catalyst (enzyme) may be "shut off" by raising the temperature. Collapse of the gel will both retard or eliminate diffusion of reactants (substrate) into the gel as well as change the microenvironment of the enzyme.

If the catalytic gel is to be useful for such reaction control, it must act reversibly. The specific enzyme activities of the gels were also studied by cycling them between 30°C and 40°C. The results are shown in Figure 13. It is evident that the enzyme activity is reversible in all of these gels. In addition, the dramatic drop in gel enzyme activity in going from 30° to 40° for the NA-100 and NA-95 gels is in sharp contrast to the NA-90 and NA-85 gels, which show a rise in activity for the same temperature changes. This is expected because the last two gels are above their LCSTs at that temperature.

immersed wet into 37° or 50° water, the release kinetics show two linear regions, a fast rate followed by a slow rate. Only when the gel is initially dry was one linear release rate observed. Therefore, thermally reversible homopolymer gels, such as lightly cross-linked poly NIPAAm, can be employed for fabricating matrix drug delivery systems which display zero-order release.

EXAMPLE VIII

GRAFTED SURFACES FOR MINIMIZING PROTEIN AND CELL ADHESION

N-isopropyl acrylamide and its copolymers with acrylamide were radiation grafted to a silicone rubber substrate. The silicone rubber (SR) substrate material used was 20 mil Silastic film (Dow Corning 500-5). The monomers employed were NIPAAm and AAm and were used as received without further purification. Irradiations were carried out in a Cobalt -60 source with the films immersed in aqueous solutions of the monomers. Cupric nitrate was added to inhibit homopolymerization in the solution. In a few cases, nitrogen atmosphere was used, but in most of the studies an air atmosphere was present. After irradiation, each film was washed in deionized water overnight, dried in a dessicator, and weighed to permit calculation of percent grafting as 100 x (weight of grafted film - original film weight + original film weight). Water contents of the grafted films were measured at different temperatures after equilibration at each temperature overnight, followed by weighing the wet film, then drying and reweighing it in the dry state. The water contents are reported as 100 x (wet weight of grafted film - dry weight of grafted film) + (wet weight of grafted film - original weight of film).

Based upon empirical studies, the following experimental conditions were selected for radiation grafting: 100 mmol/l of $Cu(NO_3)_2$, 10% total monomer concentration (NIPAAm or AAm or mixtures), air atmosphere, and an irradi-

as anticipated, the crossover at lower temperatures disappears above the LCST region (31°-33°) of poly N1PAAm.

Grafting of hydrogels onto hydrophobic substrates can yield more biocompatible surfaces while retaining the desirable physical properties of the substrate material. The rationale for grafting hydrogels is based on the hypothesis that the more hydrophilic the polymer surface, the lower the interfacial energy in the aqueous biological environment and thus the lower the thermodynamic driving force for protein adsorption and cell adhesion. When polymers are radiation grafted with gradually varying mixtures of hydrophilic and hydrophobic monomers, protein adsorption and cell adhesion often exhibit a minimum at some intermediate graft copolymer composition.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

7. The method of claim 2 including, after the step of separating, contacting the polymer gel/substance with a solution capable of releasing the bound complex from the polymer gel.

8. The method of claim 7 wherein said solution contains a composition selected from the group consisting of added acids and bases, and enzymes.

9. The method of claim 1 wherein said binding component specifically binds with said substance.

10. The method of claim 9 wherein said binding component is a receptor and said substance is selected from the group consisting of hormones, vitamins, lectins, drugs, dyes, and lipids.

11. The method of claim 9 wherein said binding component is an antibody and said substance is an antigen.

12. The method of claim 9 wherein said binding component is an enzyme and said desired substance is a substrate, inhibitor, coenzyme or cofactor.

13. The method of claim 9 wherein said binding component is selected from the group consisting of lectins, RNA, DNA (single or double stranded), ions, and stable free radicals and said desired substance is selected from the group consisting of polysaccharides, glycoproteins, RNA or DNA complementary with said binding component, oligonucleotides, proteins, steroids, chelators, complexers, ionophores, and free radicals.

14. The method of claim 1 wherein said binding component binds said desired substance nonspecifically.

22. The method of claim 1 wherein said binding component is immobilized to said polymer gel via a spacer molecule.

23. The method of claim 1 wherein said binding component is immobilized to said polymer gel by conjugating said binding component to a monomer and subsequently copolymerizing with additional monomers and cross-linking agents.

24. A method for separating a desired substance from a solution, comprising:

introducing a polymer gel that is characterized by a critical solution temperature and having a pore structure adapted to selectively retain said desired substance within the gel on the basis of size into a solution containing said desired substance; and

adjusting the temperature of the polymer gel/solution, thereby causing said gel to incorporate the desired substance and separate it from the solution.

25. The method of claim 24 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of cross-linking agent employed with the polymer to form the polymer gel.

26. The method of claim 24 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of monomer employed to form the polymer gel.

27. The method of claim 24 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of solvent employed to form the polymer gel.

hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, and copolymers thereof.

36. The method of claim 24 wherein said polymer is characterized by an upper critical solution temperature (UCST).

37. The method of claim 36 wherein the UCST polymer gel includes a polymer selected from the group consisting of polyacrylic acid and polyvinyl alcohol.

38. A method for delivering a substance into a selected environment, comprising:

incorporating a desired substance into a polymer gel characterized by a critical solution temperature by binding said substance to a binding component immobilized within said gel; and

introducing said polymer gel/substance into a selected environment containing one or more agents capable of releasing said substance from said binding component, thereby delivering said substance into the environment.

39. The method of claim 38 including, subsequent to the step of introducing, adjusting the temperature of the polymer gel to cause the gel to actively deliver the desired substance into the environment.

40. The method of claim 38 wherein said agent is selected from the group consisting of acids or bases, salts, ionic and non-ionic detergents, organic solvents, and chaotropic agents.

41. The method of claim 38 wherein said agent is a compound capable of forming a stronger complex with the desired substance than the binding component.

42. The method of claim 38 wherein said binding component specifically binds with said substance.

49. The method of claim 48 wherein said agent is selected from the group consisting of acids or bases and enzymes.

50. A method for selectively delivering a desired substance into an environment, comprising:

incorporating a desired substance into a polymer gel characterized by a critical solution temperature and having a pore structure adapted to selectively retain said desired substance within the gel on the basis of size;

introducing the polymer gel/substance into a selected environment; and

adjusting the temperature of the polymer gel/substance to selectively deliver the desired substance into the environment.

51. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of cross-linking agent employed with the polymer to form the polymer gel.

52. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of monomer employed to form the polymer gel.

53. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of solvent employed to form the polymer gel.

54. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the amount and/or composition of initiators employed to form the polymer gel.

55. The method of claim 50 wherein said pore structure is selectively adapted by adjusting the composition and/or amount of chain transfer agents employed to form the polymer gel.

FIG. 1

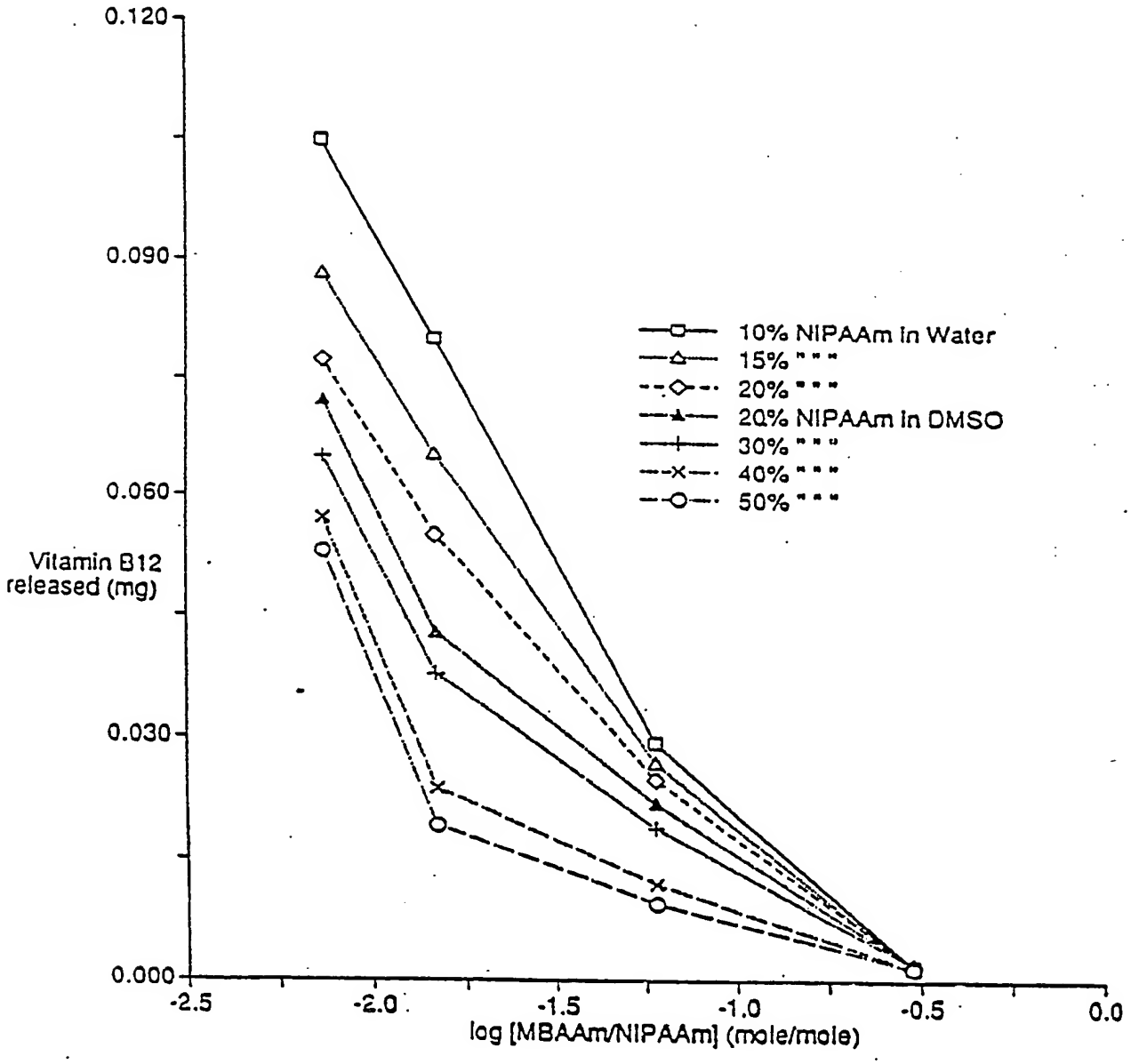

0.120
0.090
0.060
0.030
0.000
Vitamin B12 released (mg)
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
log [MBAAm/NIPAAm] (mole/mole)
10% NIPAAm in Water
15% " "
20% " "
20% NIPAAm in DMSO
30% " "
40% " "
50% " "

FIG. 2

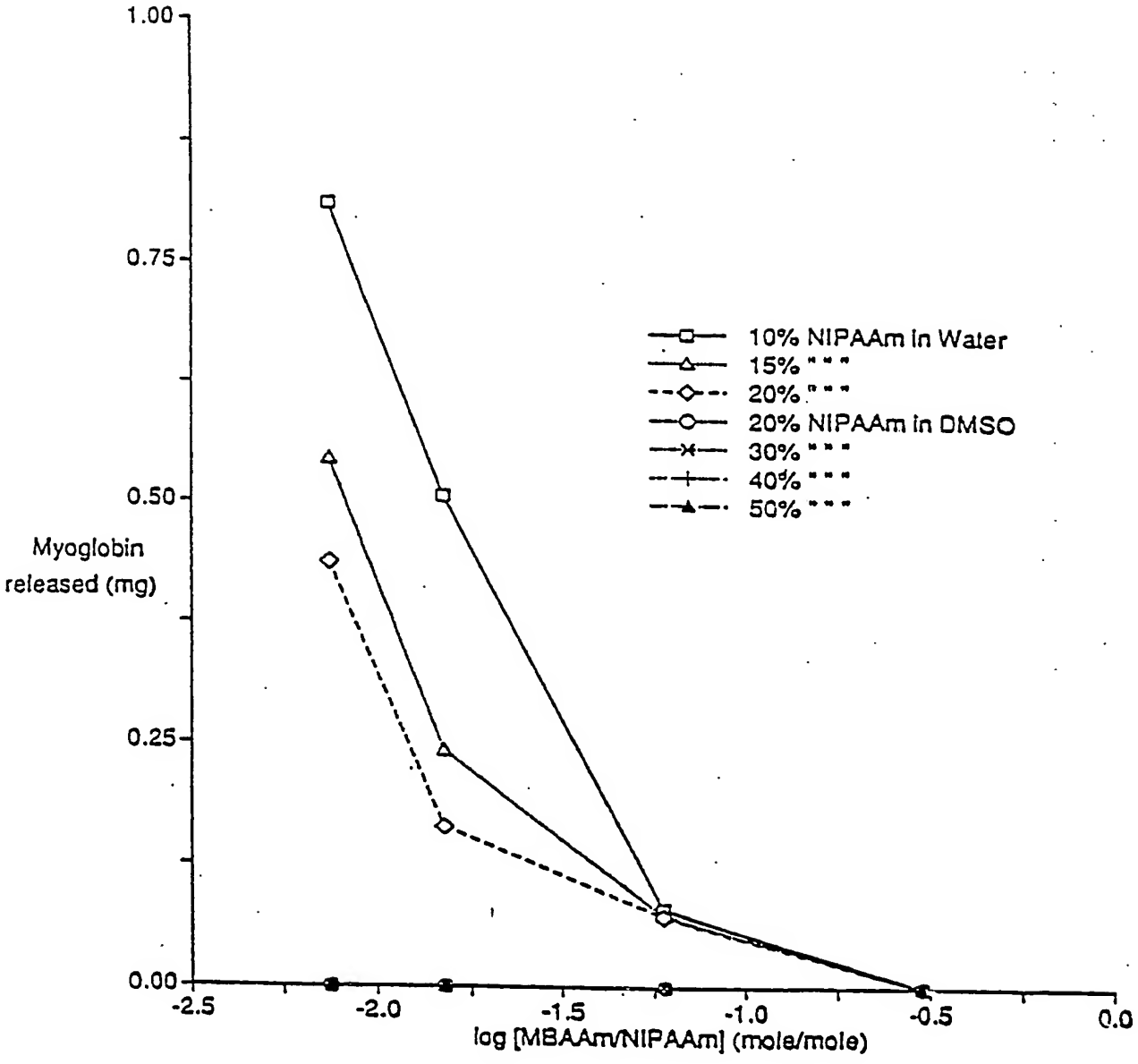
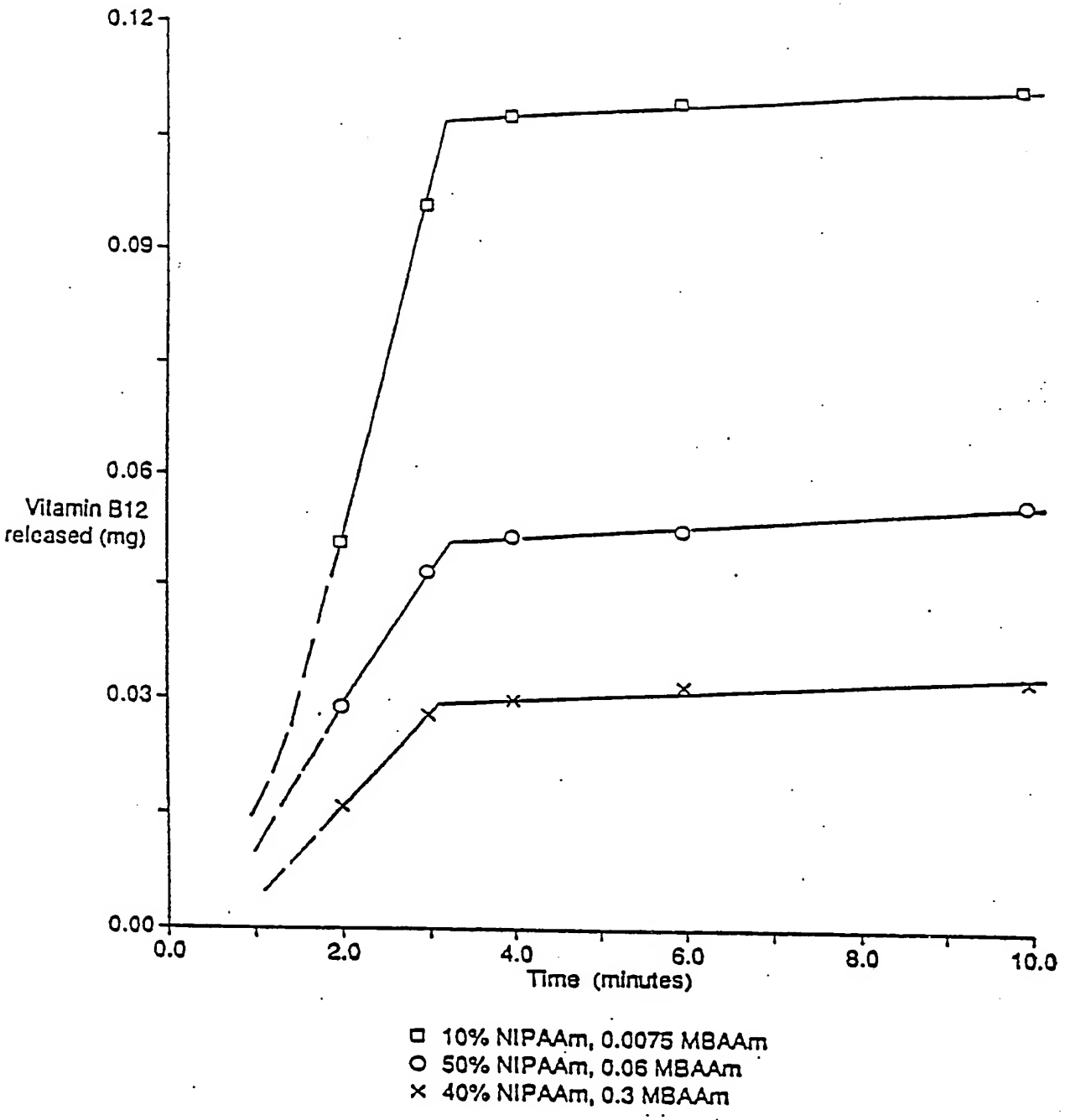

1.00
0.75
0.50
0.25
0.00
Myoglobin released (mg)
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
log [MBAAm/NIPAAm] (mole/mole)
10% NIPAAm in Water
15% " "
20% " "
20% NIPAAm in DMSO
30% " "
40% " "
50% " "

FIG. 3


0.12
0.09
0.06
0.03
0.00
Vitamin B12 released (mg)
0.0
2.0
4.0
6.0
8.0
10.0
Time (minutes)
10% NIPAAm, 0.0075 MBAAm
50% NIPAAm, 0.06 MBAAm
40% NIPAAm, 0.3 MBAAm

EFFECT OF TEMPERATURE ON WATER CONTENTS OF LOST HYDROGELS

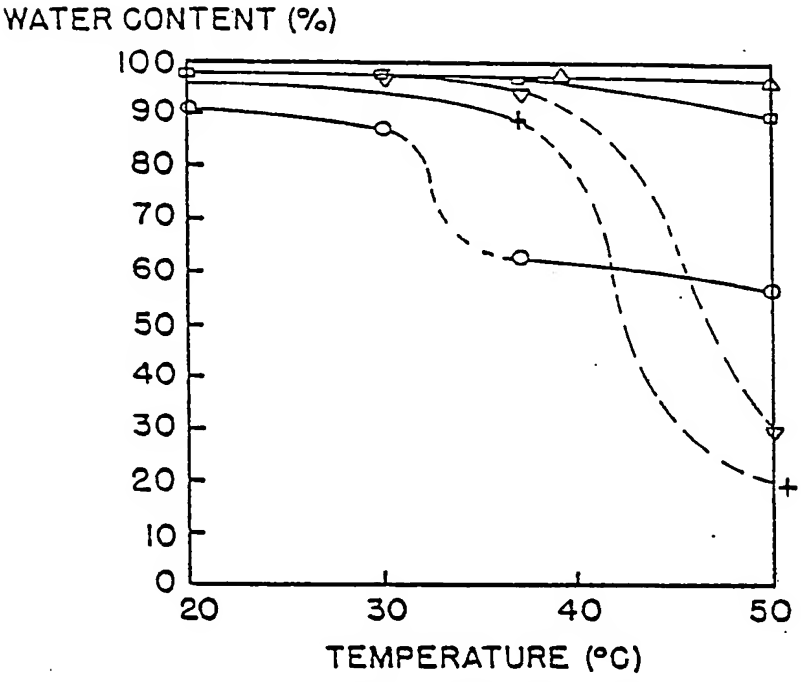


FIG. 4

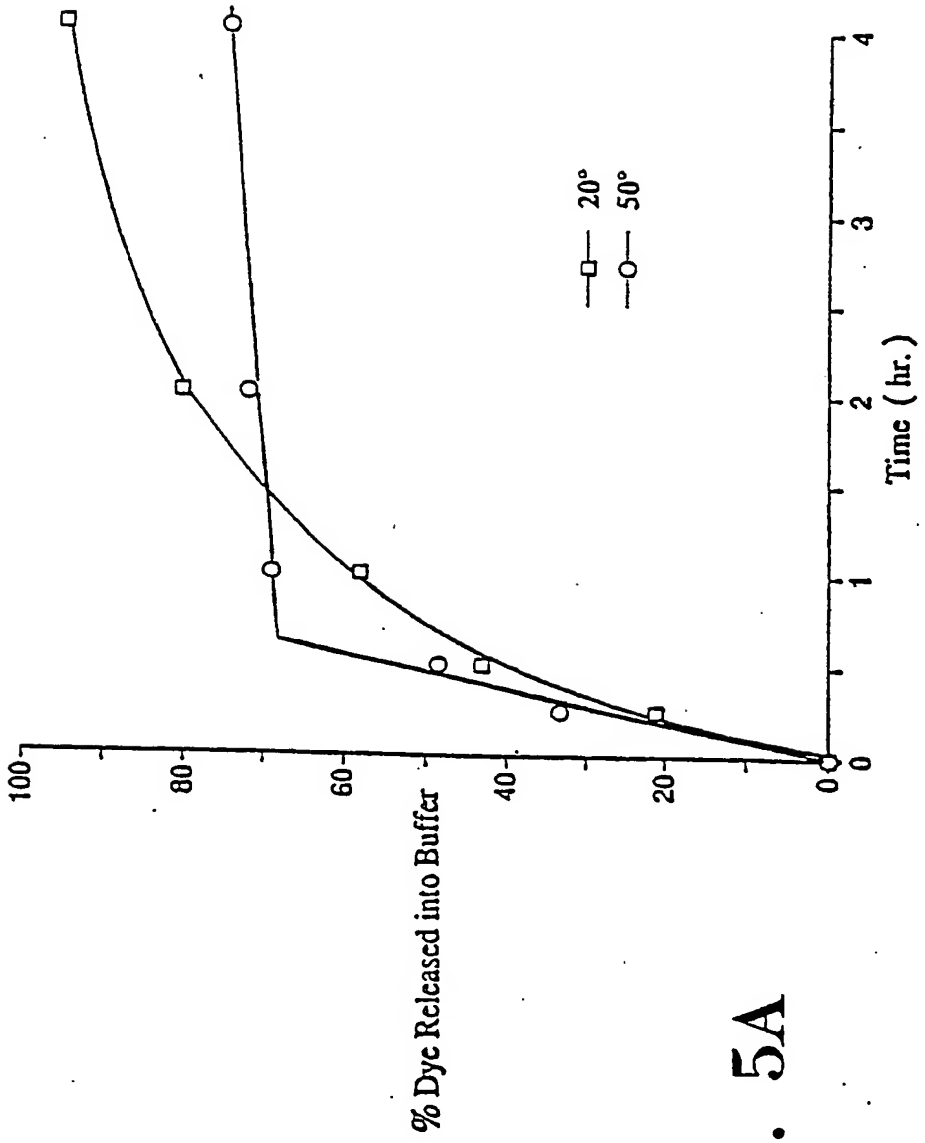


FIG. 5A

FIG. 5B

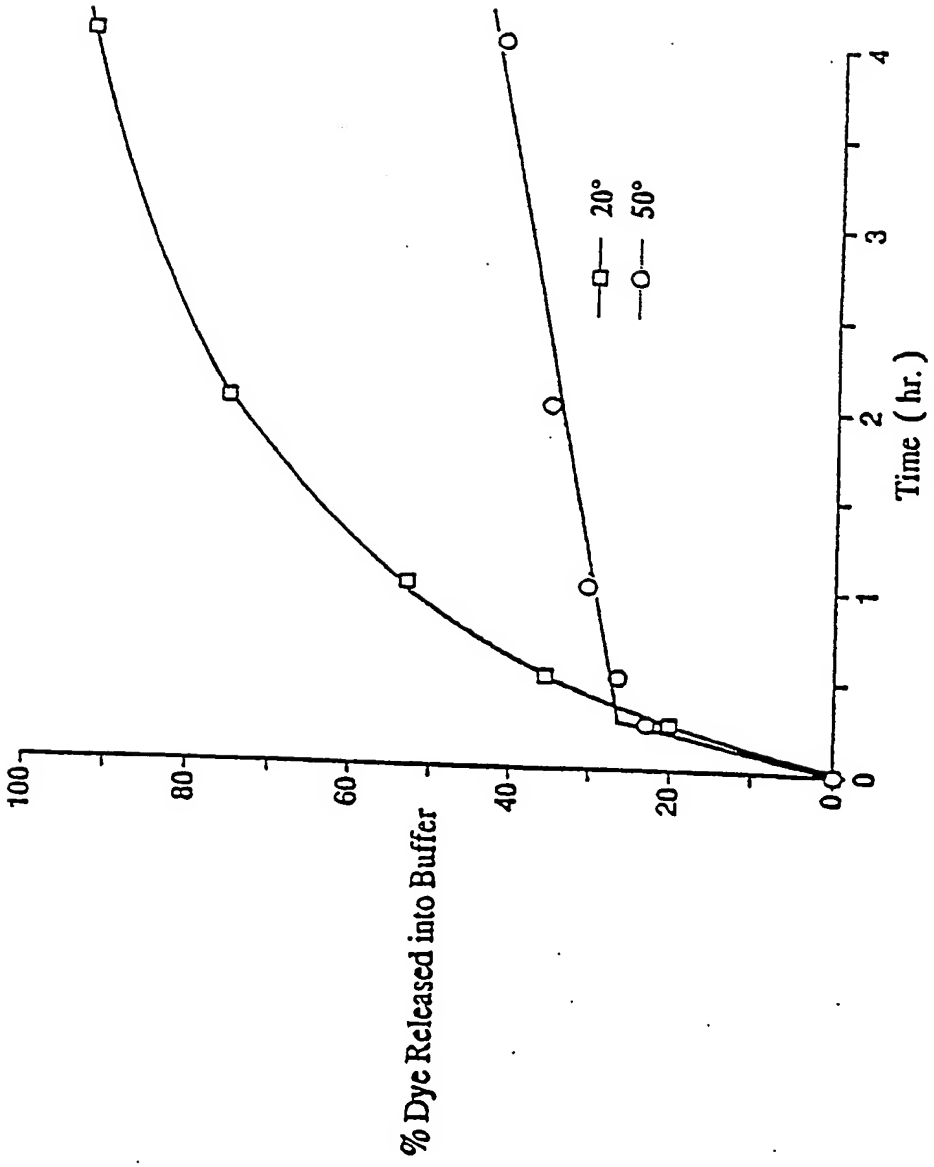
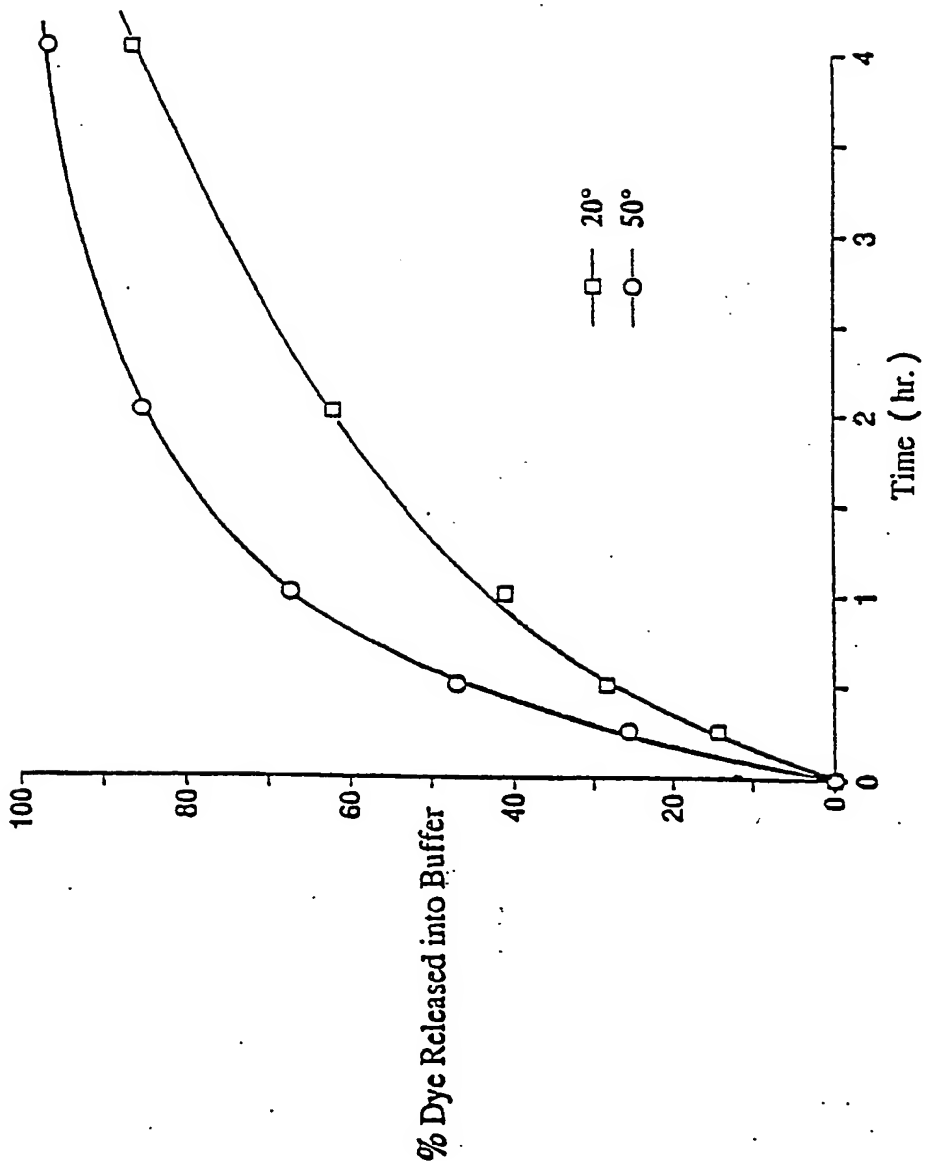


FIG. 5C



TEMPERATURE DEPENDENCE OF RELEASE RATE OF METHLENE BLUE FROM MN-I GELS IN DIFFERENT MEDIA

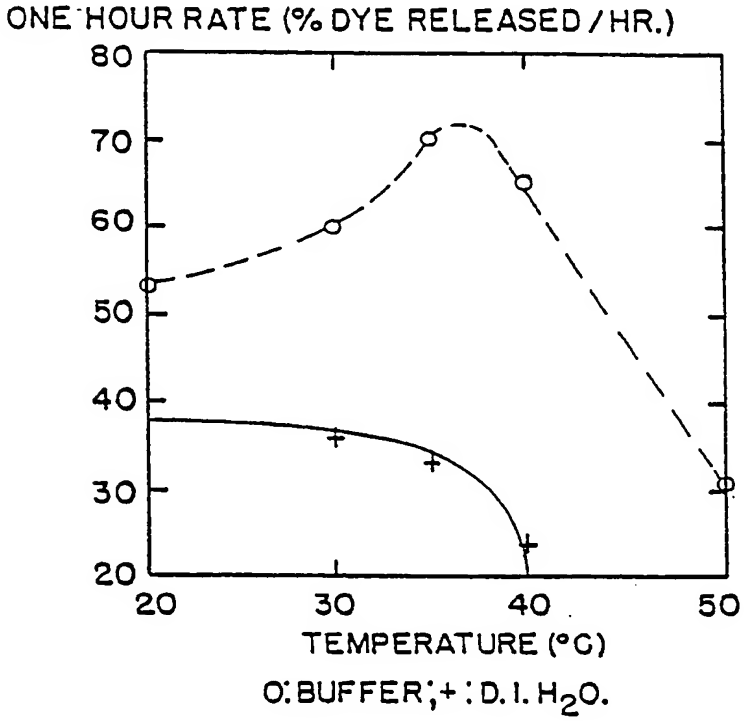
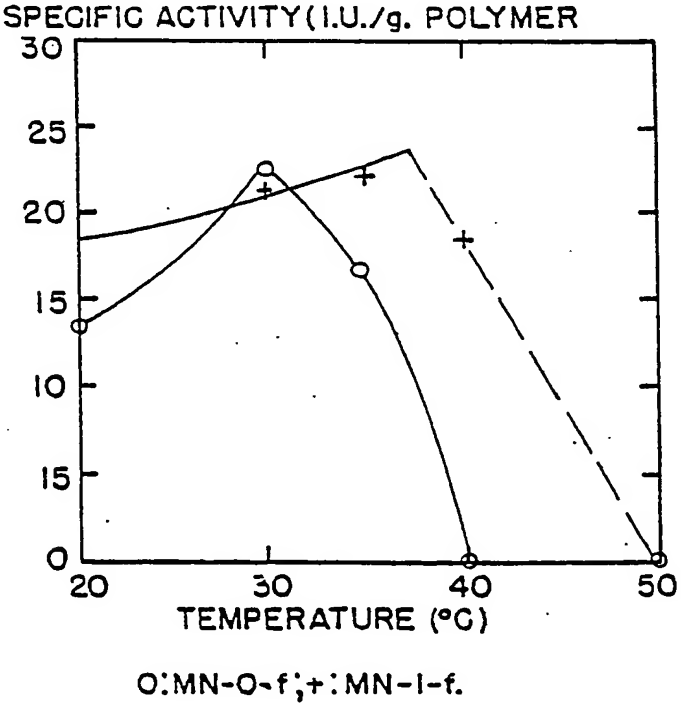


FIG. 6

THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY (FIRST RUN)

FIG. 7



THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY(THIRD RUN)

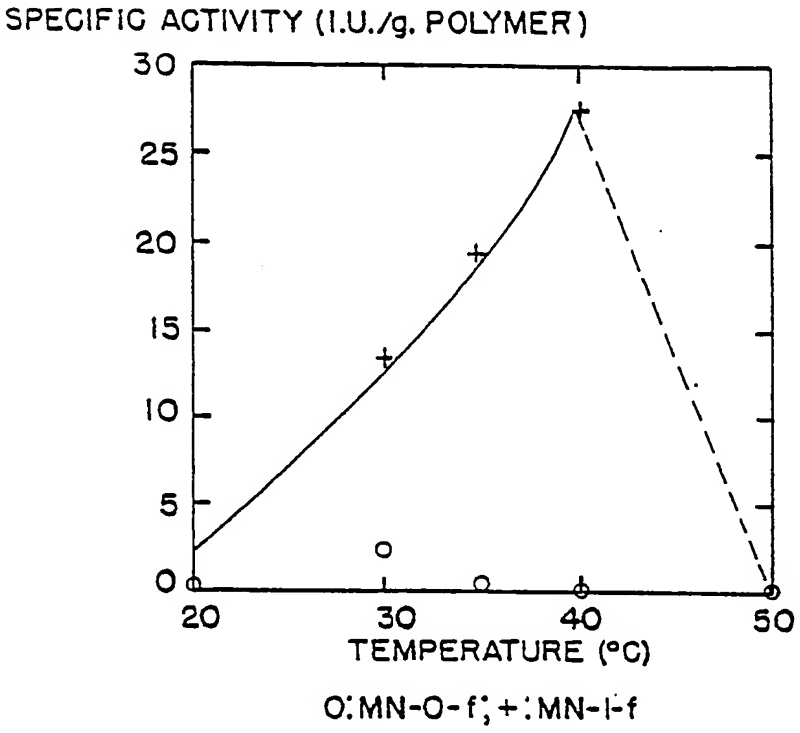


FIG. 8

THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY(FIRST RUN)

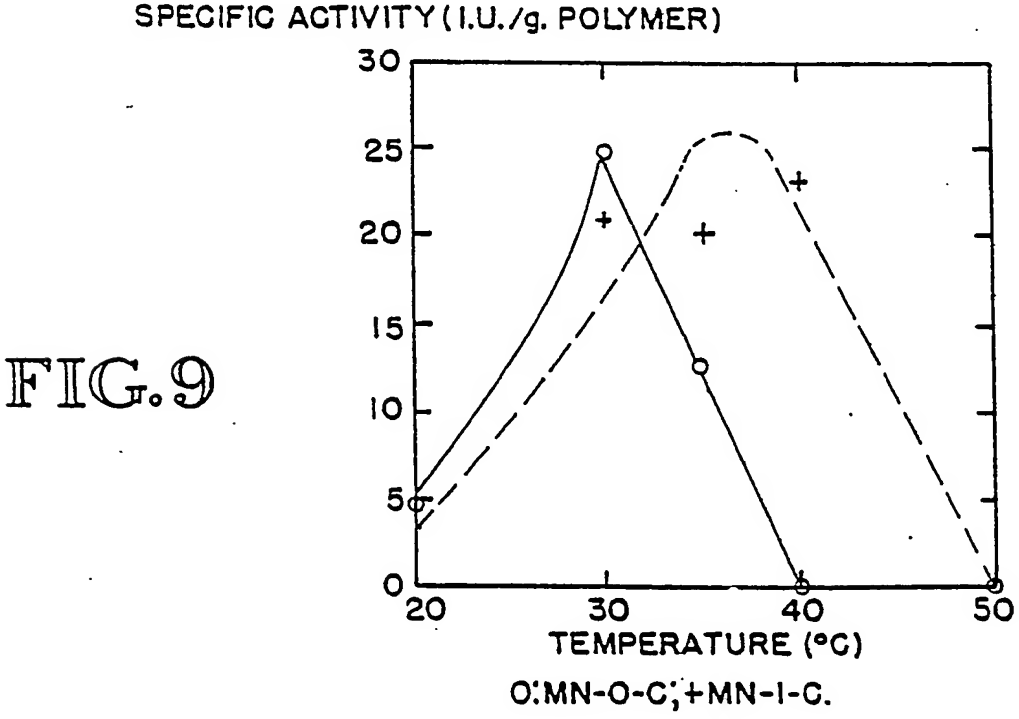


FIG. 9

THE TEMPERATURE DEPENDENCE OF SPECIFIC ACTIVITY (THIRD RUN)

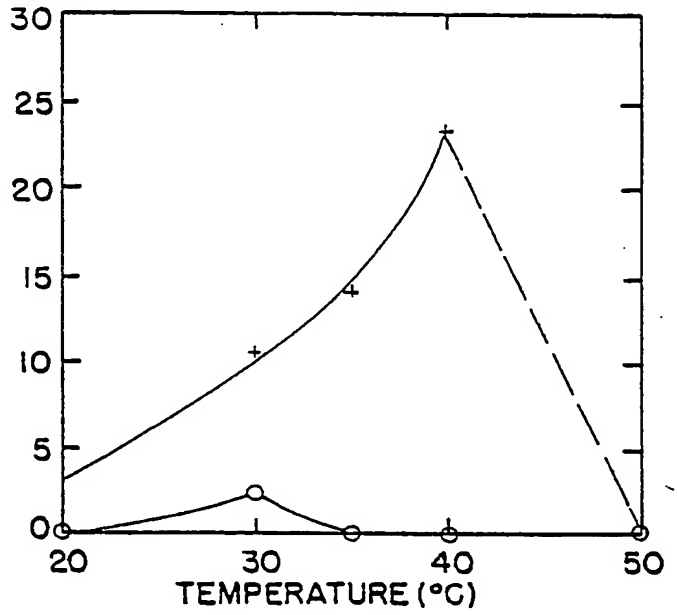


FIG. 10

O: MN-O-C; +: MN-I-C.

TEMPERATURE DEPENDENCE OF WATER CONTENT OF ASPARAGINAS, -IMMOBILIZED DISCS

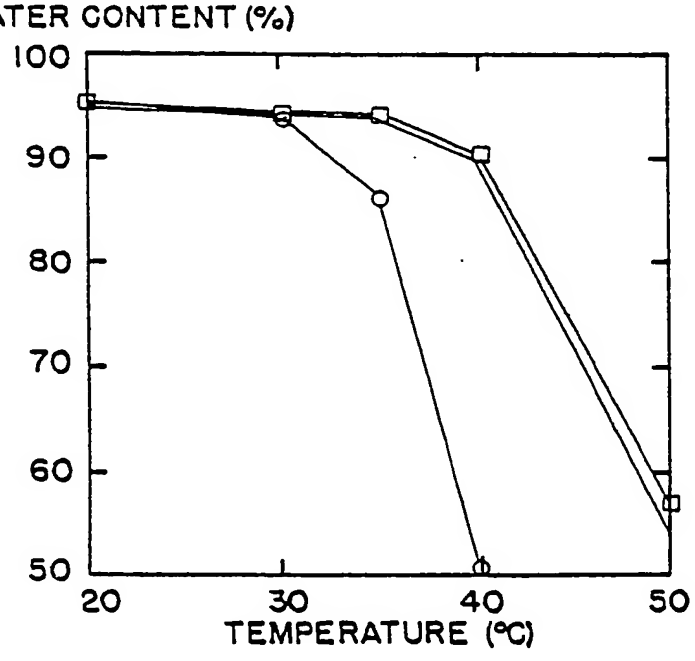
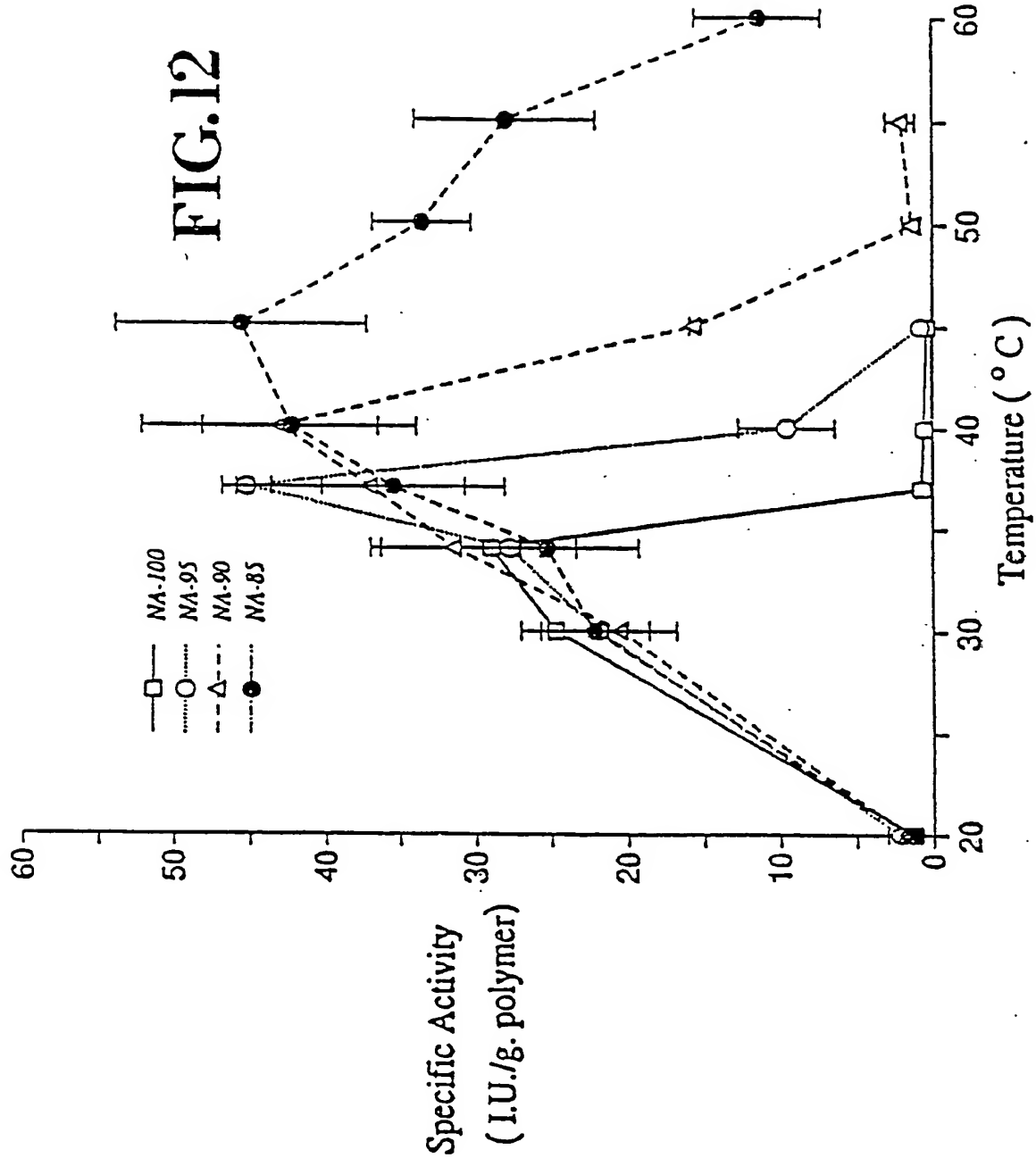


FIG. 11

O: MN-O-C; +: MN-I-C; ▽: MN-O-f; □: MN-I-f

FIG. 12


NA-100
NA-95
NA-90
NA-85
Specific Activity
(I.U./g. polymer)
Temperature (°C)
0
10
20
30
40
50
60
20
30
40
50
60

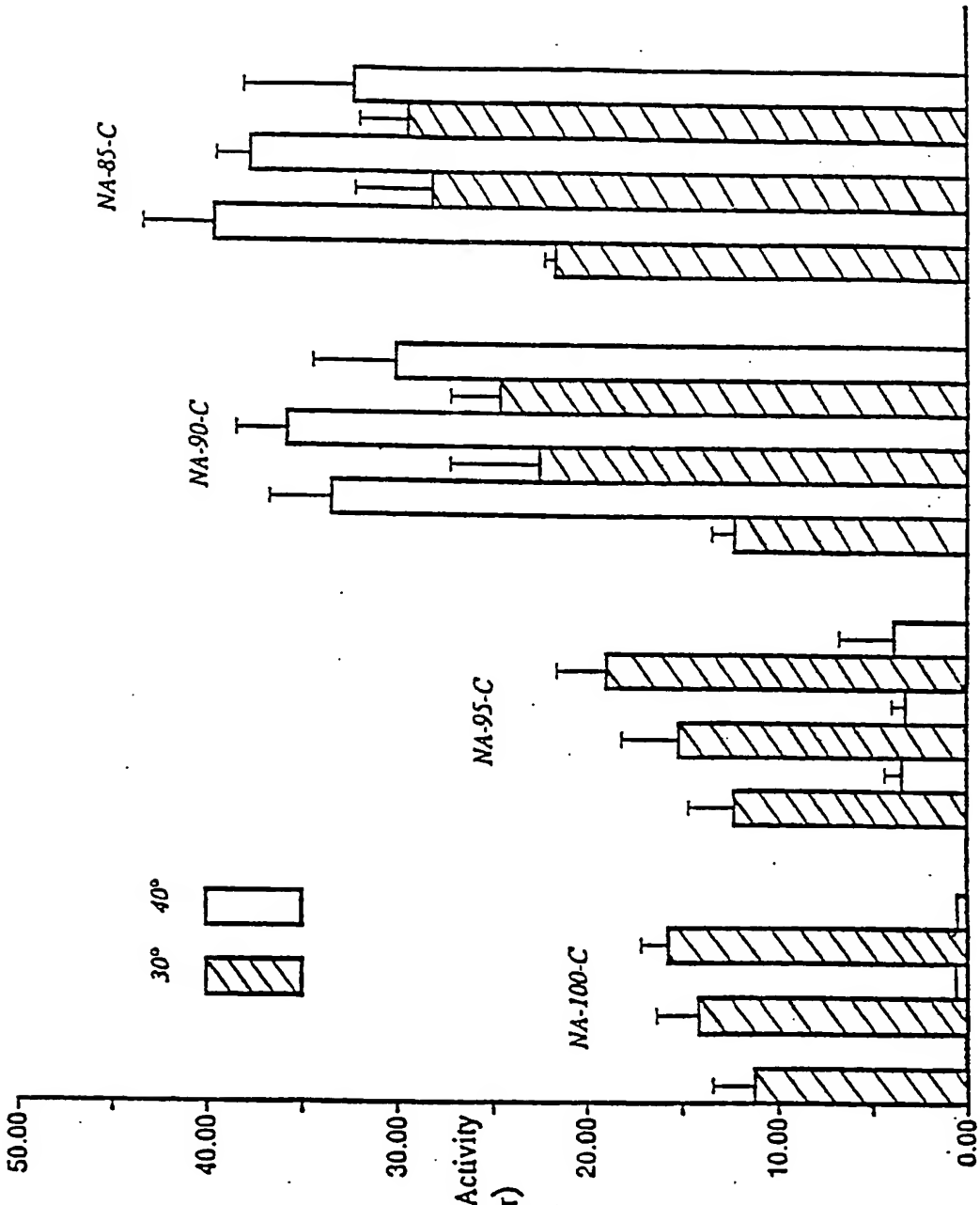
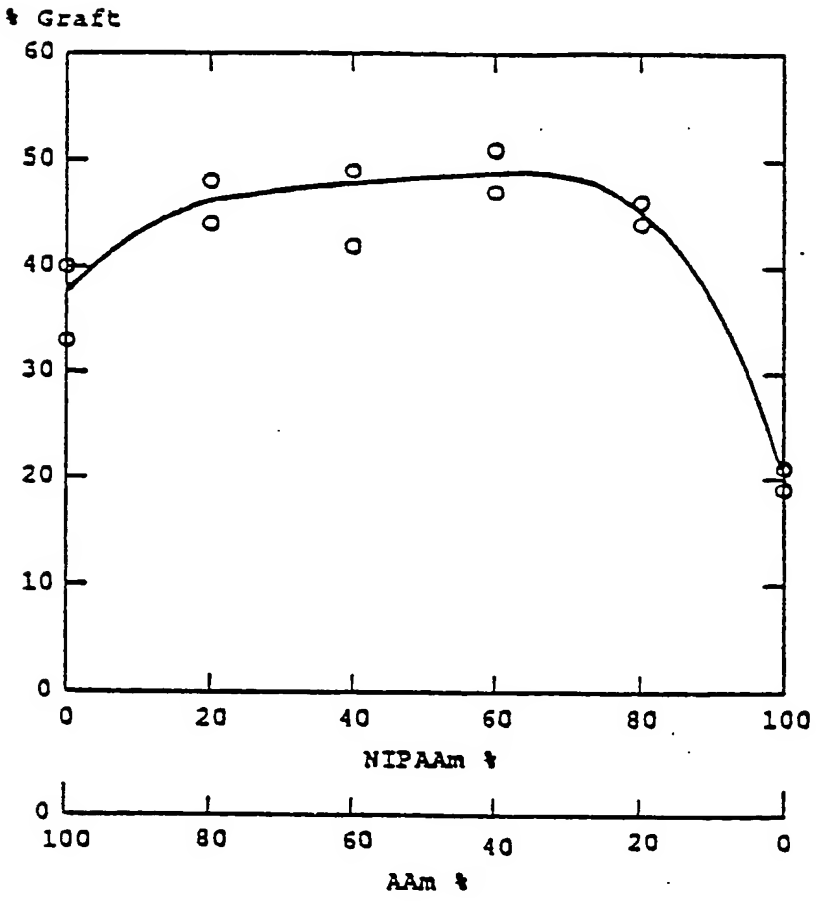

Specific Enzyme Activity
(I.U./ g. polymer)
50.00
40.00
30.00
20.00
10.00
0.00
NA-85-C
NA-90-C
NA-95-C
NA-100-C
30°
40°

FIG. 13

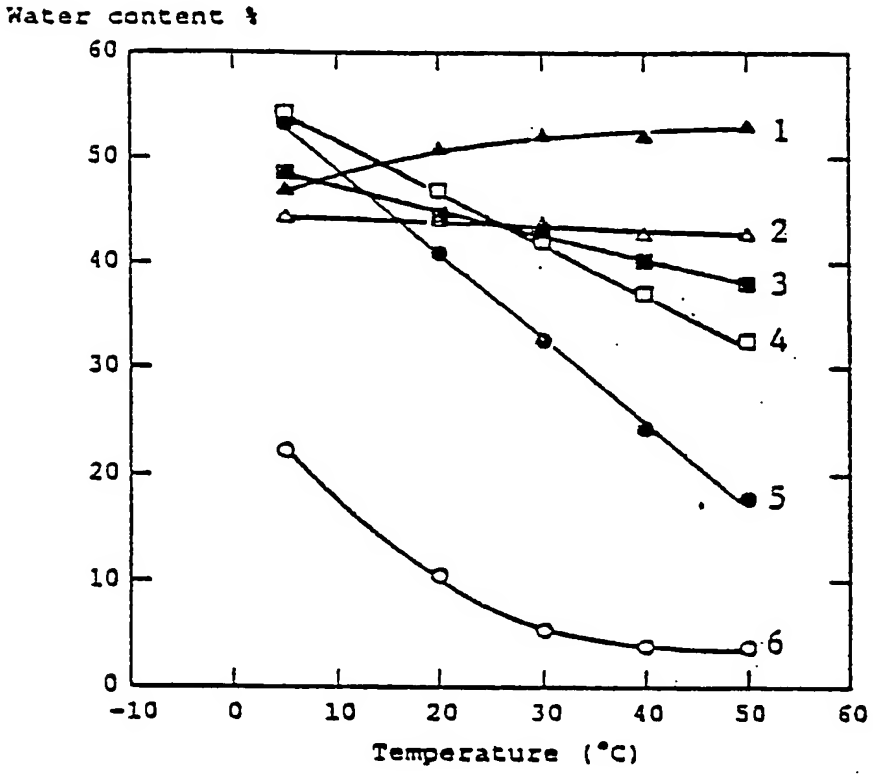
FIG. 14

Grafting NIPAAm/AAm Mixtures to Silastic



Dose: 0.68 Mrad; air atmosphere
Solutions: 100 mM $Cu(NO_3)_2$ in D.I. water
+ 10% (wt.) total monomer

Effect of Temperature on
Water Contents of Silicone Rubber
Films Grafted by NIPAAm/AAm Mixtures



Dose: 0.68 Mrad; air atmosphere.
Solutions: 100mM $Cu(NO_3)_2$ in D.I. H_2O.
+ 10% (wt) total monomer

Film no.	NIPAAm/AAm
1	0/10
2	2/8
3	4/6
4	6/4
5	8/2
6	10/0

FIG. 15

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/00886

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: B 01 D 15/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	B 01 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	GB, A, 2149803 (MITSUI TOATSU CHEMICALS) 19 June 1985 see page 1, line 50 - page 7, line 38; pages 17-18, claims 1-22	24-35,50-56
Y		1,2
Y	US, A, 3414509 (BLOCH) 3 December 1968 see column 4, line 31 - column 11, line 63	1,2
A		4,7,14,15, 38,39,48
A	EP, A, 0059598 (UNILEVER) 8 September 1982 see page 3, line 4 - page 7	4,5,9,11
Y	US, A, 4555344 (CUSSLER) 26 November 1985 see columns 5-6, claims 1-10; column 2, line 35 - column 7, line 43	24,25,32, 34
A	cited in the application	35
P,Y	Chemical & Engineering News, volume 64, no. 18, May 1986, (Washington, US), "Separation process uses swellable ./.	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th July 1987	14 SEP 1987
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/US 87/00886 (SA 17098)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/08/87

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A- 2149803	19/06/85	FR-A-	2553678	26/04/85
		NL-A-	8403204	17/05/85
		DE-A-	3436432	08/08/85
		JP-A-	60250017	10/12/85
		JP-A-	60250014	10/12/85
		JP-A-	60090010	21/05/85
US-A- 3414509		None		
EP-A- 0059598	08/09/82	WO-A-	8202818	02/09/82
		AU-A-	8142982	14/09/82
		US-A-	4490290	25/12/84
		CA-A-	1188612	11/06/85
		AU-B-	558931	12/02/87
US-A- 4555344	26/11/85	None		
WO-A- 8606492	06/11/86	AU-A-	5909986	18/11/86
		EP-A-	0221175	13/05/87